



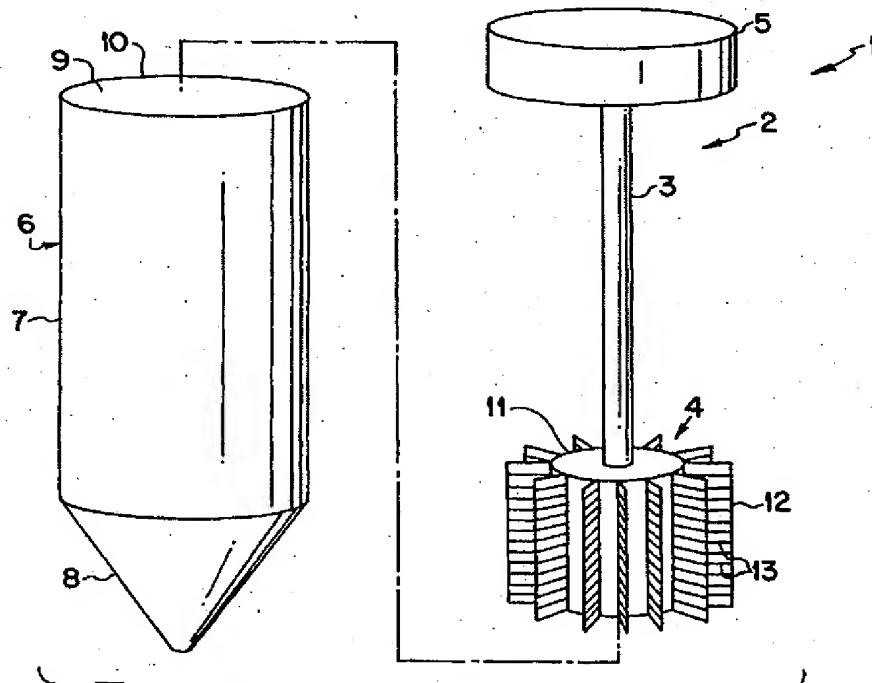
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : B01L 3/00, G01N 1/34, 33/543	A1	(11) International Publication Number: WO 00/30753 (43) International Publication Date: 2 June 2000 (02.06.00)
(21) International Application Number: PCT/US99/27741		
(22) International Filing Date: 22 November 1999 (22.11.99)		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
(30) Priority Data: 60/109,437 23 November 1998 (23.11.98) US		
(71) Applicant (for all designated States except US): USAMRIID [US/US]; United States Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, MD 21702-5011 (US).		
(72) Inventor; and		
(75) Inventor/Applicant (for US only): IBRAHIM, M., Sofi [US/US]; 1312 Willow Oak Drive, Frederick, MD 21701 (US).		Published With international search report.
(74) Agent: HARRIS, Charles, H.; United States Army Medical Research and Material Command, 504 Scott Street, Fort Detrick, MD 21702 (US).		

(54) Title: PURIFICATION METHOD AND APPARATUS

(57) Abstract

A purification apparatus (1), kit and method for purifying DNA, RNA, proteins, antigens, antibodies and cells. The apparatus (1) has a wand (2) and a reservoir tube (6). The wand (2) is made of a cap (5), a sample collection assembly (4) and an elongated shaft (3) connecting the cap (5) to the sample collection assembly (4). The sample collection (4) assembly has a series of microstructures (13) on its surface, or microparticles enclosed within it for increasing the surface area of the sample collection assembly (4). The increased surface area permits maximum exposure to and binding of target molecules thereto. The reservoir tube (6) associated with the wand (2) has one end defining an opening and a second end that is closed and preferably cone or cylindrical shaped. The cap (5) of the wand securely and sealingly fastens to the open end of the reservoir tube (6) with the shaft (3) and the sample collection (4) assembly fitting easily inside the reservoir tube.



PURIFICATION METHOD AND APPARATUS

BACKGROUND OF THE INVENTION

1. Field of the invention

This invention relates to a method, an apparatus, and kit for performing purification of nucleic acids, proteins and cells. More specifically, the invention relates to an apparatus and methods for purification and concentration of nucleic acids, proteins (e.g., antigens and antibodies) and cells without the need of centrifugation, precipitation or lengthy incubations. The apparatus and methods can be adapted to non-specific or specific capture of nucleic acids, proteins or cells in a biological or environmental samples and can be adapted for detection of the captured moiety by enzymatic colorimetric, fluorescent, luminescent or electrochemical formats with or without nucleic acids amplification.

2. Description of Related Art

Nucleic acids preparation and purification is essential to virtually all molecular biology. Most methods in use for purifying nucleic acids rely on labor-intensive organic extractions and/or centrifugation. In recent years, a new class of analytical and purification techniques have been developed which rely on the inherent biological affinities between proteins, between enzymes and their substrates, and between proteins and nucleic acids.

Affinity techniques are attractive because the desired molecules are rapidly and specifically immobilized away from the other contaminating molecules in an impure mixture, offering rapid and extensive purification or enrichment levels. Contaminating molecules are simply washed away, while target molecules remain firmly affinity-bound. Target molecules may be detached from their counterpart molecules simply by altering the environment to disfavor the affinity between the two.

In one technique, a solid phase support is used to attach target molecules from a sample, such as DNA, RNA, proteins or cells. The solid phase support can also be coated

Tris/HCl, 15 % ethanol, pH 8.5 for DNA), and the capture assembly is inserted into the reservoir, incubated at the appropriate temperature, e.g., 65°C for several minutes (or the capture assembly is subjected to the appropriate elution temperature through the thermal regulator attachment). Alternatively, it is possible to perform thermal cycling through the thermal regulator attachment while the DNA is initially bound to the capture assembly with the appropriate nucleic acids amplification buffer and reagents placed in the reservoir. The Lysis/binding, washing and elution buffer conditions may be adapted according to the sample type and the type of the nucleic acids (DNA or RNA).

However, the solid phase supports currently available do not provide vast surface area to maximize binding of molecules. In addition, they are expensive to make, and do not lend themselves to in-home or field use because of either their size or configuration. Furthermore, they do not allow the flexibility of purifying different types of molecules, e.g., nucleic acids, proteins or whole cells in a single format with the ability to capture such molecules specifically or nonspecifically, and detect such molecules (specially nucleic acids) with or without nucleic acids amplification using colorimetric, fluorescent, luminescent or electrochemical formats. The present invention, *in toto*, allows much greater flexibility and efficiency and is adaptable to future modification by, for example, incorporating thermal cycling amplification (e.g. PCR), isothermal amplification and fluorogenic, colorimetric, luminescence or electrochemical detection in the same device. The present invention also allows incorporation of specific capture molecules, e.g. dendritic (branched) oligonucleotides or peptides to further increase the capture surface area and allow the specific capture of nucleic acids, cells or proteins. In addition, the invention can be adapted to an arrayable platform to allow high throughput sample processing and detection in the same device.

What is lacking in the art is a simple, inexpensive apparatus, flexible kit and method for DNA, RNA, protein, antigen, antibody or cell purification that can be used in the field, home or laboratory with the flexibility described above. In particular, what is needed is an apparatus and method that does not require centrifugation, precipitation, lengthy incubations, or extensive equipment and that provides a massive surface area for maximum exposure to and binding of target molecules. With an increasing desire to perform rapid testing for a variety of infectious disease agents or biological markers in

The reservoir tube associated with the wand has one end defining an opening and a second end that is closed. The cap of the wand securely and sealingly fastens to the open end of the reservoir tube with the shaft and the sample collection assembly fitting easily inside the reservoir tube.

In use, for nucleic acids applications, a sample is placed inside a first reservoir tube with a lysis or denaturing solution. Then the wand is inserted into the first reservoir tube. The cap of the wand secures and seals closed the first reservoir tube. The first reservoir tube is agitated by shaking or vortexing to mix the sample with the denaturing solution. During this step, the target molecules bind to the sample collection assembly's massive surface area. The wand, which now has target molecules attached to the sample collection assembly is then removed from the first reservoir tube and inserted into a second reservoir tube which contains a wash buffer.

The second reservoir tube is then securely and sealingly closed with the cap of the wand like before. The second reservoir tube is also agitated to mix the sample with the wash buffer. The wand is then removed from the second reservoir tube and inserted into a third reservoir tube. The third reservoir tube contains an elution buffer.

The third reservoir tube; is incubated and after a short while, the DNA or RNA is purified. It can then be recovered and analysed.

A similar process is used for the capture of antigens, antibodies or cells, however different reagents or buffers are used.

Agitation or sealing is not required during the incubation steps as long as the capture assembly is in contact with the sample. However, agitation may enhance binding and sealing would help contain the sample in the reservoir and prevent accidental loss of the sample or contaminating the sample from an outside source.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a perspective view of a Purification Apparatus according to a first embodiment of the invention;

the reservoir tube for receiving the ridge 18. A tab 17 assists the user in removing the wand from the reservoir tube 6 as shown in Fig. 5.

The cap 5 is connected to one end of a shaft 3. The other end of the shaft is connected to a sample capture assembly 4. The shaft 3 is either solid or hollow and can be formed of metal or an inert synthetic material such as plastic. The sample capture assembly 4 is designed to increase surface area to a maximum to allow maximum exposure to and binding of target molecules thereto. Therefore, the sample capture assembly 4 has microstructures associated therewith, either on its surface or within it in the form of microparticles enclosed inside a mesh enclosure in a form of a "molecular sieve". If microparticles are used, further enhancements, e.g., the use of zeolitic particles, can be made to allow molecular size selection.

The sample capture assembly 4 is generally a main body 11 having microstructures on its surface in the form of cross-etched lanes, dimples, domes, pillars and/or pores. Such microstructures can be formed by tooling or etching. Preferably, cross-etched lanes in the configuration presented herein are used as microstructures and are etched to a depth of 0.001-2 mm and preferably 2 mm. The main body 11 can preferably have one or more flanges 12 protruding radially outward therefrom, wherein the microstructures 13 are on an outer surface of the flanges 12. Fig. 1a shows an enlargement of a single flange 12. Alternatively, the main body can have striations 14, wherein a cross-section of the main body 11 would reveal a jagged outer edge as shown in Fig. 3. The striations increase the surface area and preferably also have microstructures on their outer surface. The main body 11 can also be porous.

Still further, Fig. 2 shows a wand 2 having a sample capture assembly 4 that has microstructures 13a associated therewith within it in the form of microparticles enclosed inside a mesh enclosure 13b. The microparticles are made from silica-based material, polystyrene or other synthetic polymers and may be coated with a target specific surface such as specific oligonucleotides, peptides or cell receptors to capture a target DNA, RNA, protein or cell type. They are preferably about 1 to 500 μ m in diameter.

The sample collection assembly 4 may be coated with oligonucleotide probes or specific proteins to capture specific target molecules. The sample collection assembly may also be made of or coated with a material that binds non-specifically with nucleic

Rapid, universal method to isolate PCR-ready DNA using magnetic beads. BioTechniques 22:506-511.; Collin-Osdoby P., Oursler M.J., Webber D., Osdoby P. (1991). Osteoclast-specific monoclonal antibodies coupled to magnetic beads provide a rapid and efficient method of purifying avian osteoclasts. J. Bone Mine. Res. 6:1353-1365.; Cudjoe K.S., Krons R., Olsen E. (1994). IMS: A new selective enrichment technique for the detection of salmonella in foods. Int. J. Food Microbiol. 23:159-165.; Elgar G.S., Brenner S. (1992). A novel method for isolation of large insert DNA from recombinant lambda DNA. Nucleic Acids Res. 20:4667.; Gabrielsen O.S., Huet J. (1993). Magnetic DNA affinity purification of yeast transcription factor. Meth. Enzymol. 218:508-525.; Hames B.D., Higgins S.J. (1985). Nucleic acid hybridization: A practical approach. IRL Press, Oxford, England.; Hawkins R.E., Russell S.J., Winter G. (1992). Selection of phage antibodies by binding affinity. Mimicking affinity maturation. J. Mol. Biol. 226:889-896.; Boom, R., Sol, C.J., Salimans, M.M., Jansen, C.L., Wertheim-van Dillen, P.M., and van der Noordaa, J. (1990). Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol., 28(3):495-503.; Lundeberg J., Larsen F. (1995). Solid-phase technology:magnetic beads to improve nucleic acid detection and analysis. Biotechnology Annual Review 1:373-401.; Millar D.S., Withey S.J., Tizard M.L.V., Ford J.G., Hermon-Taylor J. (1995). Solid-phase hybridization capture of low abundance target DNA sequences: application to the polymerase chain reaction detection of *Mycobacterium paratuberculosis* and *Mycobacterium avium* susp. *Silvaticum*. Anal. Biochem. 226:325-330.; Vlieger A.M., Medenblik A.M.J.C., Van Gijlswijk R.P.M., Tanke H.J., Van der Ploeg M., Gratama J.W., Raap A.K. (1992). Quantitation of polymerase chain reaction products by hybridization-based assays with fluorescent, colorimetric or chemiluminescent detection. Anal. Biochem. 205:1-7.

The reservoir tube 6 serves as a reservoir for collecting samples, washing the captured nucleic acids, proteins, antibodies or antigens, and eluting the captured nucleic acid or proteins or other molecules. The reservoir tube 6 described herein has an elongated body 7 with one end having a lip 10 defining an opening 9 and a second end 8 that is closed and preferably cone shaped. The second end 8 can also be rounded or cylindrical. The cap 5 of the wand 2 securely and sealingly fastens to the open end 9 of the reservoir tube 6 with the shaft 3 and the sample collection assembly 4 fitting easily

capture assembly, elution can be achieved by adjusting the incubation temperature. After a short while, the DNA or RNA is purified. It can then be recovered and analyzed. It is also possible to perform thermal cycling while the captured DNA is bound to the capture assembly, and the elution buffer is replaced with the appropriate nucleic acid amplification buffer and reagents.

The sample can be detected by one of several methods. DNA or amplified DNA can be detected by known colorimetric, luminescent, fluorescent or electrochemical methods.

In another embodiment, the wand may further have a sensing unit 24 associated with it via a sensing contact or wire connection 25 as shown in FIG. 6 for sensing electrical or electrochemical signals emitted from the sample on the sample collection assembly, following a hybridization and/or an enzymatic reaction. Such a sensing unit would detect changes in electrical properties of bound nucleic acids or protein molecules either directly or indirectly. Direct detection can be achieved by measuring changes in current subsequent to a hybridization reaction. Indirect detection can be achieved by including in the hybridization reaction an enzyme and a substrate to drive a reduction/oxidation reaction resulting in electrical current change which can be measured by an electric current sensing device, for example. Alternatively, other indirect reaction may involve enzymatic reaction to produce colorimetric, fluorogenic or luminescence signal which can be detected with miniature optical devices such as a fluorometer or spectrometer designed to fit the closed end of the reservoir. In this embodiment, the tube would fit into such a detection device wherein the detection would take place.

The purification apparatus of the invention can be used for efficient purification of nucleic acids, proteins and cells without the need of centrifugation, precipitation or lengthy incubations. It can also be configured to allow nucleic acid amplification and detection by integrating the purification apparatus into an instrument that allows temperature cycling and detection apparatus capable of fluorescent, colorimetric, luminescent or electrochemical sensing.

EXAMPLES

Example 1

Antigen Capture and Detection

Mix 10-100 μ l of sample with 100 μ l of lysis/denaturing buffer in a 1.5 ml reservoir tube. Insert the shaft and sample capture assembly (after coating with appropriate antibody) of the wand into the reservoir tube and close the reservoir tube with the cap. Vortex the reservoir tube for about 1 minute. Incubate the reservoir tube at 37°C for about 5-15 minutes. Remove the wand, and insert the wand into a fresh reservoir tube containing 1000 μ l of blocking buffer. Vortex the reservoir tube for about 1 minute. Incubate at 37°C for about 5-15 minutes. Remove the wand and insert it into a fresh reservoir tube containing 100 μ l of conjugate solution. Remove the wand, and insert the wand into a fresh reservoir tube containing 1000 μ l of wash buffer. Shake or agitate for 1 min. Discard wash buffer and repeat the washing step. Remove the wand and insert it into a fresh reservoir tube containing 100 μ l of detection reagent. Analyze the color and determine the antigen according to a color chart. Alternatively, the color can be read by using a spectrophotometer. The detection step can also be modified to allow electrochemical, luminescent or fluorescent detection using an appropriate signal detection attachment.

Example 4

Antibody Capture and Detection

Mix 10-100 μ l of sample with 100 μ l of lysis/denaturing buffer in a 1.5 ml reservoir tube. Insert the shaft and sample capture assembly (after coating with appropriate antigen) of the wand into the reservoir tube and close the reservoir tube with the cap. Vortex the reservoir tube for about 1 minute. Incubate the reservoir tube at 37°C for about 5-15 minutes. Remove the wand, and insert the wand into a fresh reservoir tube containing 1000 μ l of blocking buffer. Vortex the reservoir tube for about 1 minute. Incubate at 37°C for about 5 minutes. Remove the wand and insert it into a fresh reservoir tube containing 100 μ l of conjugate solution. Remove the wand, and insert the wand into a fresh reservoir tube containing 1000 μ l of wash buffer. Discard the wash buffer and repeat the washing step. Remove the wand and insert it into a fresh reservoir tube containing 100 μ l of detection reagent. Analyze the color and determine the antibody.

What is claimed is:

1. A purification apparatus comprising:

a wand comprising a cap, a sample collection assembly and an elongated shaft connecting said cap to said sample collection assembly, said sample collection assembly having microstructures for increasing the surface area of the sample collection assembly; and

a reservoir tube having a lip defining an opening, wherein said cap securely and sealingly fastens to said lip of said reservoir tube with said shaft and said sample collection assembly inside said reservoir tube.

2. The purification apparatus of claim 1, wherein said microstructures are selected from the group consisting of cross-etched lanes, dimples, pillars and pores.

3. The purification apparatus of claim 1, wherein said sample collection assembly comprises a main body having one or more flanges, wherein said microstructures are on an outer surface thereof for binding target molecules.

4. The purification apparatus of claim 3, wherein said microstructures are selected from the group consisting of cross-etched lanes, dimples, pillars and pores.

5. The purification apparatus of claim 1, wherein said sample collection assembly comprises a main body having an outer surface, wherein said microstructures are on said outer surface for binding target molecules.

6. The purification apparatus of claim 1, wherein said sample collection assembly comprises a mesh outer surface and wherein said microstructures comprise microparticles enclosed within said mesh outer surface.

17. The purification apparatus of claim 1, wherein said reservoir tube comprises an elongated body having a lip defining an opening at a first end and a cone or cylindrical shaped second end.

18. The purification apparatus of claim 1, wherein said wand further comprises a heating unit associated with said wand for heating the sample collection assembly.

19. The purification apparatus of claim 1, wherein said wand further comprises a sensing unit associated with said wand for sensing electrical or electrochemical signals through said sample collection assembly.

20. The purification apparatus of claim 1, wherein said reservoir has associated therewith a detecting unit comprising an optical device or spectrometer.

21. A purification kit comprising:
packaged in association together,
a wand comprising a cap, a sample collection assembly and an elongated shaft connecting said cap to said sample collection assembly, said sample collection assembly having a surface containing microstructures for increasing the surface area of the sample collection assembly; and
a plurality of reservoir tubes or microtiter plate modules wherein each of said reservoir tube or module has a lip defining an opening, wherein said cap securely and sealingly fastens to a said lip of said reservoir tubes or modules with said shaft and said sample collection assembly inside said reservoir tube or module.

22. The purification kit of claim 21, wherein said microstructures are selected from the group consisting of cross-etched lanes, dimples, pillars and pores.

23. The purification kit of claim 21, wherein said sample collection assembly comprises a main body having an outer surface, wherein said microstructures are on said outer surface for binding target molecules.

collection assembly, said sample collection assembly having microstructures for increasing the surface area of the sample collection assembly;

securely and sealingly closing said first reservoir tube with said cap of said wand with said shaft and said sample collection assembly inside said first reservoir tube;

agitating said first reservoir tube to mix said sample with said denaturing solution; thereby binding said DNA or said RNA to said sample collection assembly;

removing said wand from said first reservoir tube and inserting said wand into a second reservoir tube; said second reservoir tube containing a wash buffer;

securely and sealingly closing said second reservoir tube with said cap of said wand with said shaft and said sample collection assembly inside said second reservoir tube;

agitating said second reservoir tube to mix said sample with said wash buffer;

removing said wand from said second reservoir tube and inserting said wand into a third reservoir tube; said third reservoir tube containing an elution buffer;

incubating said third reservoir tube; and

recovering purified DNA or RNA from said third reservoir tube.

32. The method of claim 31, wherein said sample capture assembly comprises a main body having one or more flanges for binding target molecules.

33. The method of claim 32, wherein said flanges further comprises an outer surface having microstructures selected from the group consisting of cross-etched lanes, dimples, pillars and pores.

34. The method of claim 31, wherein said sample collection assembly comprises outer surface having said microstructures, wherein said microstructures are selected from the group consisting of cross-etched lanes, dimples, pillars and pores.

35. The method of claim 31, wherein said sample collection assembly comprises a mesh outer surface with microparticles enclosed within said mesh outer surface.

- h) removing said wand from said second reservoir tube and inserting said wand into a third reservoir tube; said third reservoir tube containing a conjugate solution;
- i.) removing said wand from said third reservoir tube and inserting said wand into a forth reservoir tube; said forth reservoir tube containing a wash buffer and washing several times;
- j) capturing said antigen on said coating of said capture assembly.

41. The method of claim 40, further comprising removing said wand from said forth reservoir tube and inserting in a fifth reservoir tube; said fifth reservoir tube containing a detection agent; and

detecting said antigen by a method selected from the group consisting of colorimetric, fluorescent, luminescent and electrochemical methods.

42. The method of claim 40, wherein said microstructures are selected from the group consisting of cross-etched lanes, dimples, pillars and pores.

43. The method of claim 40, wherein said sample collection assembly comprises a main body having an outer surface, wherein said microstructures are on said outer surface for binding target molecules.

44. The method of claim 40, wherein said sample collection assembly comprises a main body having one or more flanges associated therewith, wherein said flanges have microstructures on an outer surface thereof for binding target molecules.

45. The method of claim 44, wherein said microstructures are selected from the group consisting of cross-etched lanes, dimples, pillars and pores.

46. The method of claim 40, wherein said sample collection assembly comprises a mesh outer surface with microparticles enclosed within said mesh outer surface.

- h) removing said wand from said second reservoir tube and inserting said wand into a third reservoir tube; said third reservoir tube containing a conjugate solution;
- i) removing said wand from said third reservoir tube and inserting said wand into a forth reservoir tube; said forth reservoir tube containing a wash buffer and washing several times; and
- j) capturing said antibody on said coating of said capture assembly.

52. The method of claim 51, further comprising removing said wand from said forth reservoir tube and inserting it into a fifth reservoir tube; said fifth reservoir tube containing a detection agent; and

detecting said antibody by fluorescent, colorimetric, luminescent or electrochemical methods.

53. The method of claim 51, wherein said microstructures are selected from the group consisting of cross-etched lanes, dimples, pillars and pores.

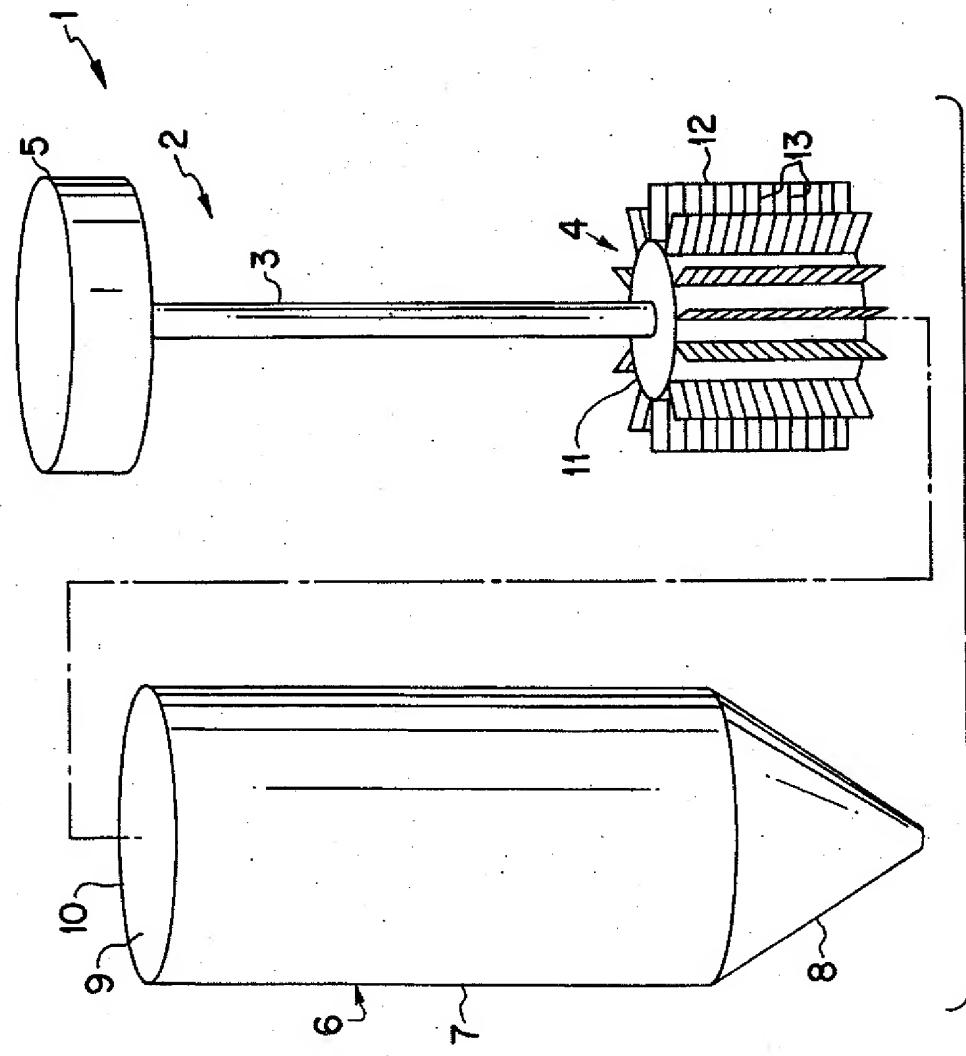
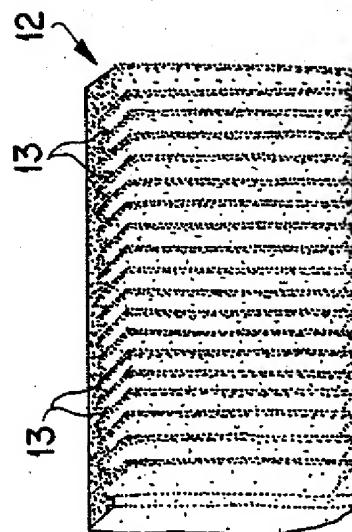
54. The method of claim 51, wherein said sample collection assembly comprises a main body having an outer surface, wherein said microstructures are on said outer surface for binding target molecules.

55. The method of claim 51, wherein said sample collection assembly comprises a main body having one or more flanges associated therewith, wherein said flanges have microstructures on an outer surface thereof for binding target molecules.

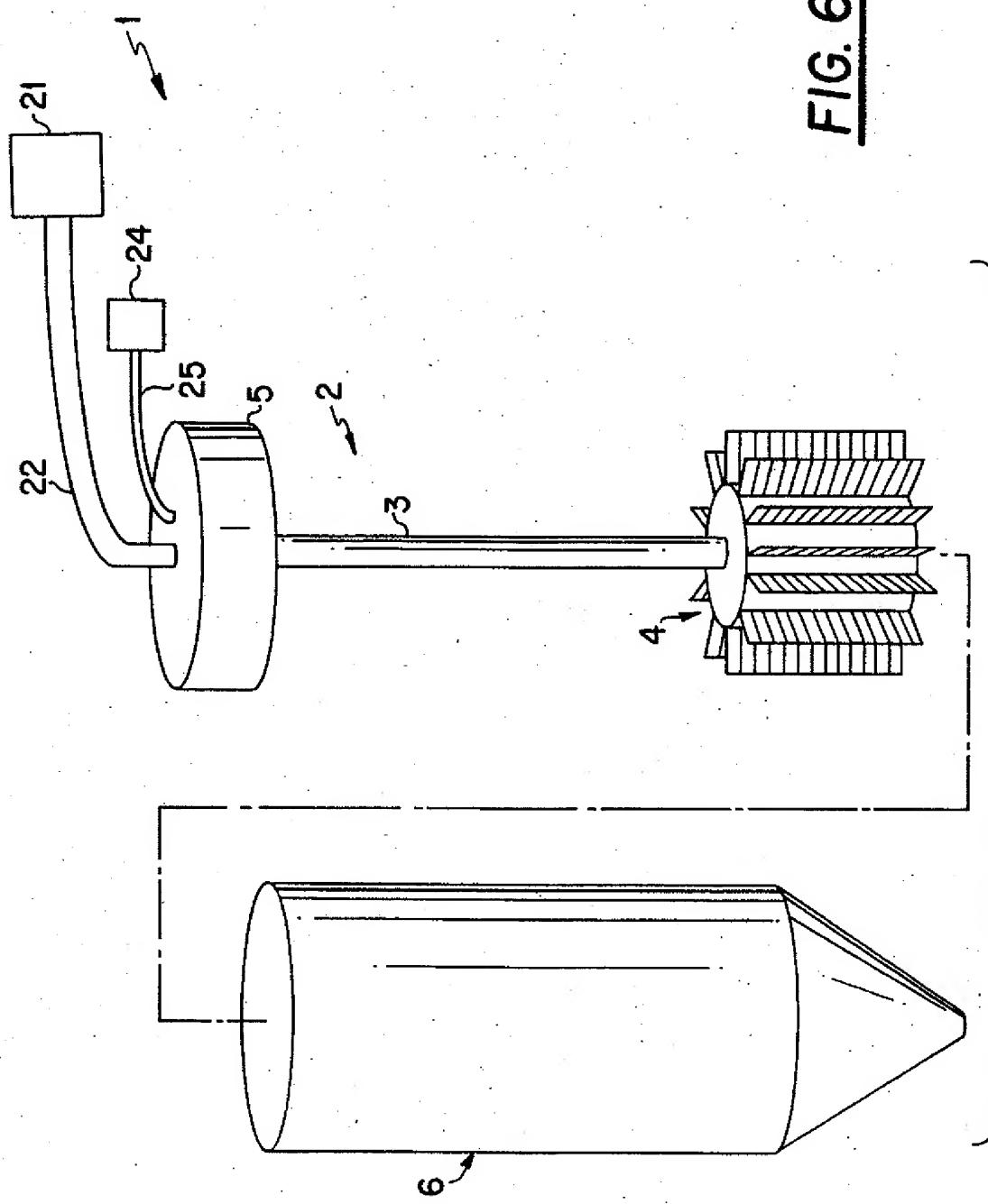
56. The method of claim 55, wherein said microstructures are selected from the group consisting of cross-etched lanes, dimples, pillars and pores.

57. The method of claim 51, wherein said sample collection assembly comprises a mesh outer surface with microparticles enclosed within said mesh outer surface.

1/3



3/3

FIG. 6

INTERNATIONAL SEARCH REPORT

Internat'l Application No

PCT/US 99/27741

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4 657 869 A (RICHARDS JAMES C ET AL) 14 April 1987 (1987-04-14) abstract; figures 1,2 column 2, line 15 -column 4, line 27	1,21,31, 40,51,62
A	US 4 789 628 A (NAYAK P N) 6 December 1988 (1988-12-06) abstract; figures 3,4 column 6, line 19 -column 7, line 61	1,21,62